

Figure 1: Human POSH Coding Sequence (SEQ ID NO:1) (part 1)

ATGGATGAATCAGCCTTGTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGA  
AGGTCTTGCCTTGCCAGCATACGTTTTTGAAGCGATGTTTGTCTGGGGATCGTAGGTTCTCGAAATGAAC  
CAGATGTCCCGAGTGCAGGACTCTTGTGGCTCGGGTGTGAGGAGCTCCAGTAACATCTTGCTGGTC  
AGACTTCTGGATGGCATCAAACAGAGGCTTGGAAACCTGGTCTGGTGGGGGAAGTGGGACCAACTGCA  
CAAATGCATTAAGGTCTCAGAGCAGCACTGTGGCTAATTGTAGCTCAAAGATCTGCAGAGCTCCCAGGG  
CGGACAGCAGCCTCGGGTGAATCCTGGAGCCCCCAGTGAGGGGTATACCTCAGTTACCATGTGCCAAA  
GCGTTATACAACATATGAAGGAAAAGAGCCTGGAGACCTTAAATTCAGCAAAGGCGACATCATCATTTTGC  
GAAGACAAGTGGATGAAAATTGGTACCATGGGGAAGTCAATGGAATCCATGGCTTTTTTCCCCACCAACTT  
TGTGCAGATTATTAAACCGTTACCTCAGCCCCACCTCAGTGCAAAGCACTTTATGACTTTGAAGTGAAA  
GACAAGGAAGCAGACAAAGATTGCCTTCCATTTGCAAAGGATGATGTTCTGACTGTGATCCGAAGAGTGG  
ATGAAAACCTGGGCTGAAGGAATGCTGGCAGACAAAATAGGAATATTTCCAATTTTCATATGTTGAGTTTAA  
CTCGGCTGCTAAGCAGCTGATAGAATGGGATAAGCCTCCTGTGCCAGGAGTTGATGCTGGAGAATGTTCC  
TCGGCAGCAGCCCAGAGCAGCACTGCCCCAAAGCACTCCGACACCAAGAAGAACACCAAAAAGCGGCAC  
CCTTCACCTTCCCTCAGTATGGCCAACAAGTCCTCCCAGGCATCCAGAACCGCCACTCCATGGAGATCAG  
CCCCCTGTCTCATCAGCTCCAGCAACCCCACTGCTGCTGCACGGATCAGCGAGCTGTCTGGGCTCTCC  
TGCACTGCCCCCTTCTCAGGTTTCATATAAGTACCACCGGGTTAATTGTGACCCCGCCCCAACGAGCCAG  
TGCAACTGGCCCCCTCGTTTACTTTCCCATCAGATGTTCCCTACCAAGCTGCCCTTGGAACTTTGAATCC  
TCCTCTTCCACCACCCCTCTCCTGGCTGCCACTGTCTGCTTCCCTCCACACCACAGGCGCCACCGCCGC  
GCTGCTGCTGCTGGAATGGGACCGAGGCCATGGCAGGATCCACTGACCAGATTGCACATTTACGGCCGC  
AGACTCGCCCCAGTGTGTATGTTGCTATATATCCATACACTCCTCGGAAAGAGGATGAAGTAGAGCTGAG  
AAAAGGGGAGATGTTTTTAGTGTTTGAAGCGCTGCCAGGATGGCTGGTTCAAAGGGACATCCATGCATACC  
AGCAAGATAGGGGTTTTCCCTGGCAATTATGTGGCACCAGTCACAAGGGCGGTGACAAATGCTTCCCAAG  
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AGGGCTGCCAGAAAGCTCCAGGGAATGGCGTGGCTGGGAGTCCAGTGTGTCCCCGAGCTGTGGTA  
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ACCAGGCCCGCAATGCTGTGAGGACAGTTGCAGCGCACAAACCAGGAACGCCCCACGGCAGCAGTGACACC  
CATCCAGGTACAGAATGCCGCCGGCCTCAGCCCTGCATCTGTGGGCCTGTCCCATCACTCGCTGGCCTCC  
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ATGGCTGGTTCAAAGGCACATTACAACGTAATGGGAAAACCTGGCCTTTTCCAGGAAGCTTTGTGGAAAA  
CATATGA

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Figure 2: Human POSH Amino Acid Sequence (SEQ ID NO:2) (part 2)

MDESALLDLLECPVCLERLDASAKVLPCQHTFCKRCLLGIVGSRNELRCPECRTLVGSGVEELPSNILLV  
RLLDGIKQRPWKPGPGGGSGTNCNALSQSSTVANCSSKDLQSSQGGQOPRVQSWSPVVRGIPQLPCKAK  
ALYNYEGKEPGDLKFSKGDIIILRRQVDENWYHGEVNGIHGFFPTNFVQIIKPLPQPPPQCKALYDFEVK  
DKEADKDCLPFAKDDVLTVIRRVNENWAEGMLADKIGIFPISYVEFNAAKQLIEWDKPPVPGVDAGECS  
SAAAQSSTAPKHSDTKKNTKKRHSFTSLTMANKSSQASQNRHSMEISPPVLISSSNPTAAARISELSGLS  
CSAPSQVHISTTGLIVTPPPSSPVTGTPSFTFPPSDVPYQAALGTLNPPPLPPPPLLAATVLASTPPGATAA  
AAAAGMGPRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGEMFLVFERCQDGFKGTSMT  
SKIGVFPNGYVAPVTRAVTNASQAKVPMSTAGQTSRGVTMVSPSTAGGPAQKLQNGVAGSPSVVPAAVV  
SAAHIQTSPQAKVLLHMTGQMTVNQARNVARTVAAHNQERPTAAVTPIQVQNAAGLSPASVGLSHHSLAS  
PQPAPLMPGSATHTAAISISRASAPLACAAAAPLTSPSITSASLEAEPSGRIVTVLPGLPTSPDSASSAC  
GNSSATKPKDKSKKEKKGLLKLKLLSGASTKKRKPVSPPASPTLEVELGSAELPLQGAVGPPELPPGGGHGRA  
GSCPVDGDGPVTTAVAGAALAQDAFHRKASSLDSAVPIAPPPRQACSSLGPVLNESRPVVCERHRVVVSY  
PPQSEAELELKEGDIVFVHKKREDGWFKGTLQRNGKTGLFPGSFVENI

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGAGAGACACTGCGAGCGGCGAGCGGGTGGGGCCGCATCTGCATCAGCCGCCGAGCCGCTGCGGGGC  
CGCGAACAAAGAGGAGGAGCCGAGGCGCGAGAGCAAAGTCTGAAATGGATGTTACATGAGTCATTTTAAG  
GGATGCACACAACATATGAACATTTCTGAAGATTTTTCTCAGTAAAGTAGATAAAGATGGATGAATCAGC  
CTTGTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGAAGGTCTTGCCTTGC  
CAGCATACGTTTTGCAAGCGATGTTTGTGGGGATCGTAGGTTCTCGAAATGAACTCAGATGTCCCGAGT  
GCAGGACTCTTGTGGCTCGGGTGTGAGGAGCTTCCAGTAACATCTTGTGGTTCAGACTTCTGGATGG  
CATCAAACAGAGGCCTTGGAAACCTGGTCTGTGGGGGAAGTGGGACCAACTGCACAAATGCATTAAGG  
TCTCAGAGCAGCACTGTGGCTAATTGTAGCTCAAAGATCTGCAGAGCTCCAGGGCGGACAGCAGCCTC  
GGGTGCAATCCTGGAGCCCCCAGTGAGGGGTATACCTCAGTTACCATGTGCCAAAGCGTTATACAACTA  
TGAAGGAAAAGAGCCTGGAGACCTTAAATTCAGCAAAGGCGACATCATCATTTTTCGGAAGACAAGTGGAT  
GAAAATTGGTACCATGGGGAAGTCAATGGAATCCATGGCTTTTTCACCACTTGTGTCAGATTATTA  
AACCGTTACCTCAGCCCCCAGTGTGCAAGCACTTTATGACTTTGAAGTGAAGACAAGGAAGCAGA  
CAAAGATTGCCCTTCCATTTGCAAAGGATGATGTTCTGACTGTGATCCGAAGAGTGGATGAAAACCTGGCT  
GAAGGAATGCTGGCAGACAAAATAGGAATATTTCCAATTTTCATATGTTGAGTTTAACTCGGCTGCTAAGC  
AGCTGATAGAAATGGGATAAGCCTCCTGTGCCAGGAGTTGATGCTGGAGAATGTTCTCGGCAGCAGCCCA  
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ACTATGGCCAAACAGTCTCCTCCAGGCATCCAGAACCGCCACTCCATGGAGATCAGCCCCCTGTCTCTCA  
TCAGCTCCAGCAACCCCACTGTGCTGCACGGATCAGCGAGCTGTCTGGGCTCTCTGCAGTGGCCCTTC  
TCAGTTTCATATAAGTACCACCGGTTAATTGTGACCCCGCCCCAAGCAGCCAGTGAACAATGGCCCC  
TCGTTTACTTTTCCCATCAGATGTTCCCTACCAAGCTGCCCTTGGAACTTTGAATCCTCTCTCCACAC  
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TTTCTCTGGCAATTATGTGGCACCAAGTCAAAAGGGCGGTGACAAATGCTTCCCAAGCTAAAGTCCCTATG  
TCTACAGCTGGCCAGACAAGTCGGGGAGTGACCATGGTCAGTCCCTTCCACGGCAGGAGGGCCTGCCCAGA  
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GCTGTGAGGACAGTTGCAGCGCACAAACAGGAACGCCCCACGGCAGCAGTGACACCCATCCAGGTACAGA  
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TCTGATGCCAGGCTCAGCCACGCACACTGCTGCCATCAGTATCAGTCGAGCCAGTGGCCCTCTGGCCTGT  
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GACACTGAAGAAGCTTAAATCACTTCAACAAAGTAGCACAAGCAGTTTAAACAGAAAGAGCACAT  
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TATAAGAAACACTGTGAAAAGTTCATATTCATCCATTGTGATTCTTTCCACAGTCTTGCATGTATTACT  
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GTACTCAGAATTGACCAATTACAGAGGTGTAATAATAACAGTGTCTCTCTCTACCCCAAAGCCACTA

-to be continued

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGACCAAGGTCTCTTCAGTGCACTCGCTCCCTCTCTGGCTAAGGCATGCATTAGCCACTACACAAGTCA  
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CTGTGACTGTGGAGCTCTGGAAGGCTTGGTGGGAGTGAATTTGCCACACCTTACAATTGTGGCAGGATC  
CAGAAGAGCCTGTCTTTTATATCCATTCTTGATGTTCATTGGCCTCTCCACCGATTTTATTACGGTGC  
CACGCAGTCATGGATCTGGGTAGTCCGGAAAACAAAAGGAGGGAAGACAGCCTGGTAATGAATAAGATCC  
TTACCACAGTTTTCTCATGGGAAATACATAATAAACCTTTTCATCTTTTTTTTTTCCCTTTAAGAATTAA  
AACTGGGAAATAGAAACATGAACGAAAAGTCTTGCAATGACAAGAGGTTTCATGGTCTTAAAAAGATAC  
TTTATATGGTTGAAGATGAAATCATTCCTAAATTAACTTTTTTTTTTAAAAAAAACAATGTATATTATGT  
TCCTGTGTGTTGAATTTAAAAAAAATACTTTACTTTGGATATTCATGTAATATATAAAGGTTTGGTG  
AAATGAACTTTAGTTAGGAAAAAGCTGGCATCAGCTTTCATCTGTGTAAGTTGACACCAATGTGTCATAA  
TATTCTTTATTTTGGGAAATTAGTGTATTTTATAAAAATTTTAAAAAGAAAAAAGACTACTACAGGTTAA  
GATAATTTTTTTTACCTGTCTTTTCTCCATATTTTAAGCTATGTGATTGAAGTACCTCTGTTCATAGTTTC  
CTGGTATAAAGTTGGTTAAAATTTTCATCTGTTAATAGATCATTAGGTAATATAATGTATGGGTTTTCTAT  
TGGTTTTTTTGCAGACAGTAGAGGGAGATTTTGTAAACAAGGGCTTGTACACAGTGATATGGTAATGATAA  
AATTGCAATTTATCACTCCTTTTCATGTTAATAATTTGAGGACTGGATAAAAGGTTTCAAGATTAAATTT  
TGATGTTCAAACCTTTGT

Figure 4: 5' cDNA fragment of human POSH (public gi:10432611; SEQ ID NO:4)

ctgagagacactgcgagcggcgagcgcggtggggccgcatctgcatcagccgcgcgagccgctgcggggc  
cgcgaacaaagaggaggagccgaggcgcgagagcaaagtctgaaatggatgttacatgagtcattttaag  
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cagcagctgctccactgacttccccaaagcatcaccagtgtctcttgaggctgagcccagtgggccgat  
agtaccgttctccctggactccccacatctcctgacagtgttcatcagcttgtgggaacagttcagca  
accaaaccagacaaggatagc



Figure 6: 3' mRNA fragment of hPOSH (public gi:7959248; SEQ ID NO:6)

atttcatatgttgagtttaactcggctgctaagcagctgatagaatgggataagcctcctgtgccaggag  
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 aagggttgttacacagtgatattggttaataaattgcaatttatcactccttttcatgttaataatt  
 tgaggactggataaaaaggtttcaagataaaatttgactgttcaaacctttgt

Figure 7: C terminus protein fragment of hPOSH (public gi:7959249; SEQ ID NO:7)

ISYVEFN SAAKQLIEWDKPPVPGVDAGECSSAAQSS TAPKHS DTKKNTKKRHSFTSLTMANKSSQASQN  
RHSMEISPPVLISSSNPTAAARISELSGLSCSAPSQVHISTTG LIVTPPPSSPVTTGPSFTFP SDVPYQA  
ALGTLNPPPLPPPPLLAATV LASTPPGATAAAAAAGMGRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRK  
EDELELRKGEMFLVFERCQDGF KGTSMHTSKIGVFP GNYVAPVTRAVTNASQAKVPMSTAGQTSRGVTM  
VSPSTAGGPAQKLQGN GVAGSPSVVPAAVVSAAHIQTS PQA KVL LHM TGQMTV NQARNAV RTVA AHNQER  
PTAAVTPIQVQNAAGLSPASVGLSHHSLAS PQPAPLMPGSATHTA AISISRASAPLACAAAAPLTSPSIT  
SASLEAEPSGRIVTVLPGLPTSPDSASSACGNSSATKPKDKSKKEKKGLLKL LSGASTKRKPRVSPASP  
TLEVELGSAELPLQGA VGPELPPGGGHGRAGSCPVDGDGPVTTAVAGAA LAQDAFHRKASSLDSAVPIAP  
PPRQACSSLGPVLNESRPVVCERHRVVVSYPQSEAELELKEGDIVFVHKKREDGWFKGTLQRNGKTGLF  
PGSFVENI



Figure 8: Human POSH full mRNA, Annotated Sequence (part 1)

```

---- - gi|10432611|dbj|AK021429.1|AK021429 Homo sapiens cDNA
FLJ11367 fis, clone HEMBA1000303, highly similar to Mus musculus
Plenty of SH3s (POSH) mRNA

```

```

----- gi|7959248|dbj|AB040927.1|AB040927 Homo sapiens mRNA for
KIAA1494 protein, partial cds

```

--:-- - Both hPOSH and KIAA1495

~~2-2-1~~ - Ring Domain

100-441144 - SH3 Domian

 - start codon and stop codon of predicted ORF

[illegible]

-to be continued

Figure 8: Human POSH full mRNA, Annotated Sequence (part 2)

TTGTGGACTTCCAGATGGTCAGGAGATGAGCAAAGGATTGGTATGTGACTCTGATGCCCCAGCACAGTTA  
CCCCAGCGAGCAGAGTGAAGAAGATGTTTGTGTGGGTTTGTAGTCTGGATTCGGATGTATAAGGTGTG  
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TTGGATTATGATTTTAAGAAATTATTAATTTATGAAATGATAGGTAAGGAGAAGCTGGATTATCTCCTGT  
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TGGGTTTTTAATTTTCTAGAAATGAAGTGACTGAAACAATGAGAAAGAATACAGCACAAACCTTGAACAA  
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Figure 9: Domain Analysis of Human POSH

Domain Name	begin	end	E-value
<u>RING</u>	12	52	1.06e-08
<u>SH3</u>	137	192	2.76e-19
<u>SH3</u>	199	258	4.84e-15
<u>low complexity</u>	366	384	-
<u>low complexity</u>	390	434	-
<u>SH3</u>	448	505	2.40e-19
<u>low complexity</u>	547	563	-
<u>low complexity</u>	652	668	-
<u>low complexity</u>	705	729	-
<u>SH3</u>	832	888	1.47e-14

Figure 10: Diagram of Human POSH Nucleic Acids

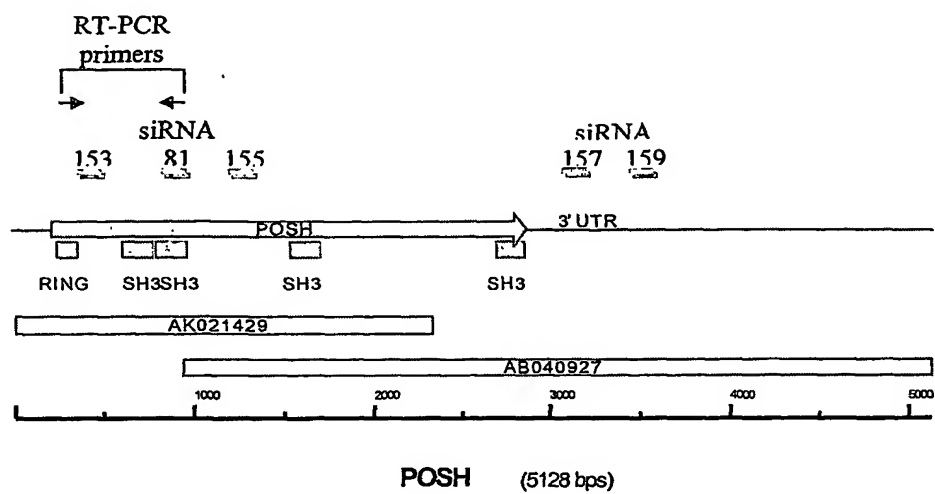


Figure 11: Reduction in Full Length POSH mRNA by siRNA Duplexes

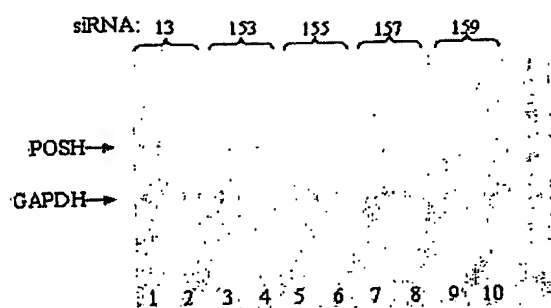


Figure 12: POSH Affects Release of VLP from Cells

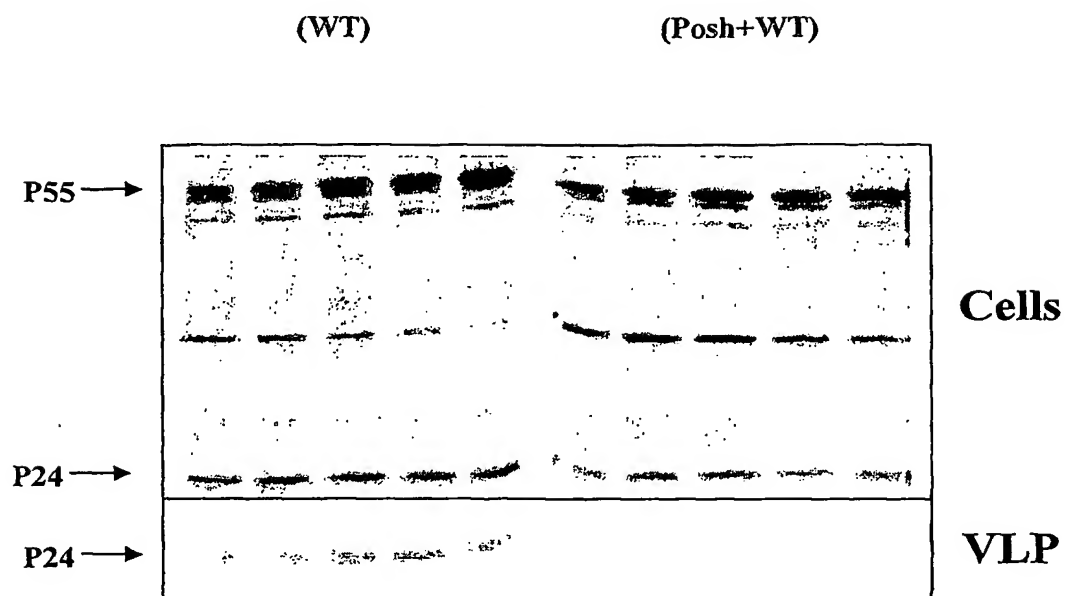
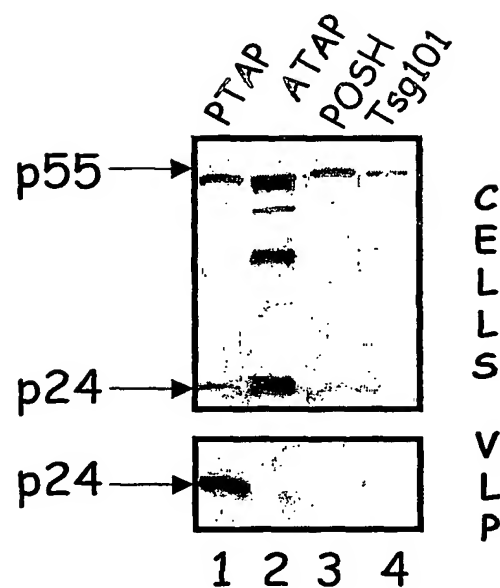


Figure 13: Release of VLP from Cells at Steady State



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GGGAATACCTCAGTTACCCTGTGCGCAAGCATATATAACTACGAAGGAAAAGAGCCCGGAGACCTTAA  
GTTACGAAAGGCGACACCATCTTCTGCGCCGACAGGTGGATGAGAATTTGGTACACGGGGAAGTCAGC  
GGGGTCCACGGCTTTTTCCCACTAACTTCTGTGCAGATCATCAAACCTTTACCTCAGCCCCGCGCTCAGT  
GCAAAGCACTTTACGACTTTGAAGTGAAGACAAGGAAGCTGACAAAGATTGCCTTCCCTTCGCAAAGGA  
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Figure 15: Mouse POSH Protein sequence (Public gi: 10946922; SEQ ID NO: 9)

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Figure 16: *Drosophila melanogaster* POSH mRNA sequence (public gi:17737480; SEQ ID NO:10)

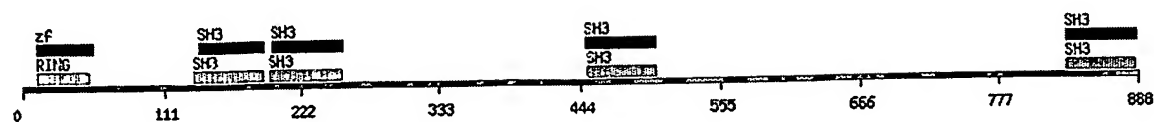
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Figure 17: *Drosophila melanogaster* POSH protein sequence (public gi:17737481; SEQ ID NO:11)

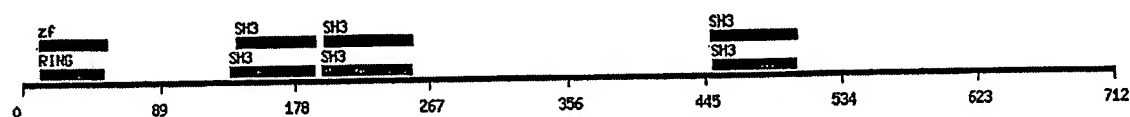
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Figure 18: POSH Domain Analysis

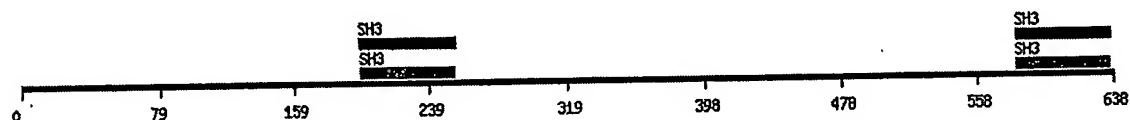
hPOSH protein sequence :



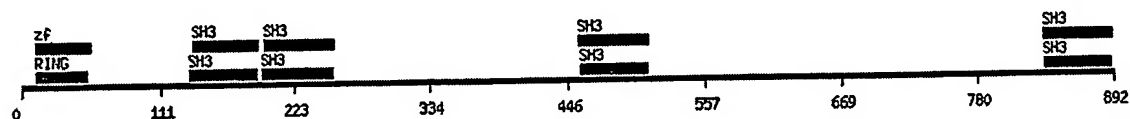
N terminus protein fragment of hPOSH (public gi:10432612):



C terminus protein fragment of hPOSH (public gi:7959249):



Mouse POSH Protein sequence (Public gi: 10946922):



Drosophila melanogaster POSH protein sequence (public gi:17737481)

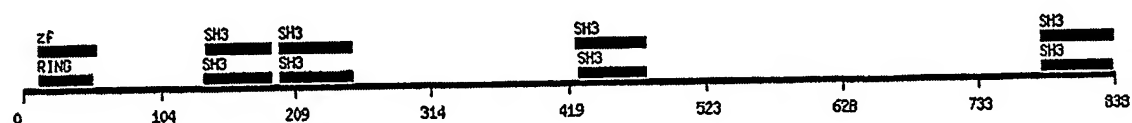


Figure 19: Human POSH has ubiquitin ligase activity

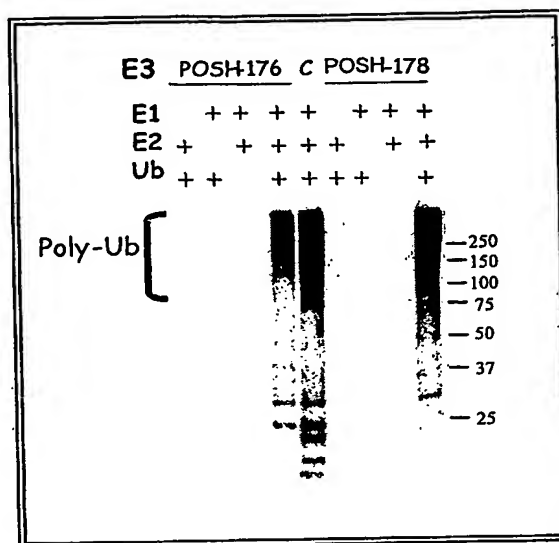


Figure 20.

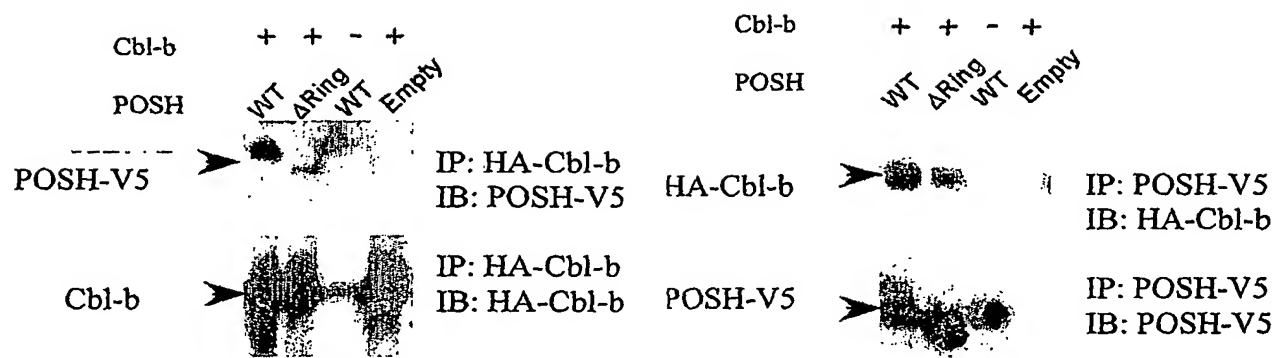


Figure 21. PLD activity in medium of transfected cells

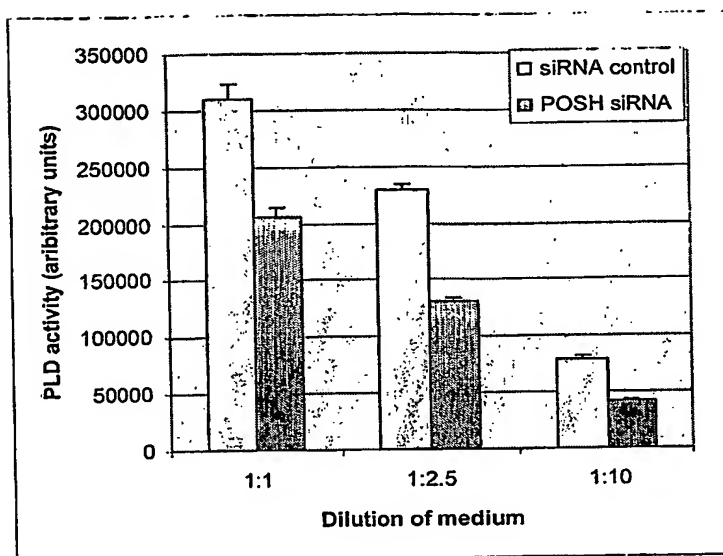


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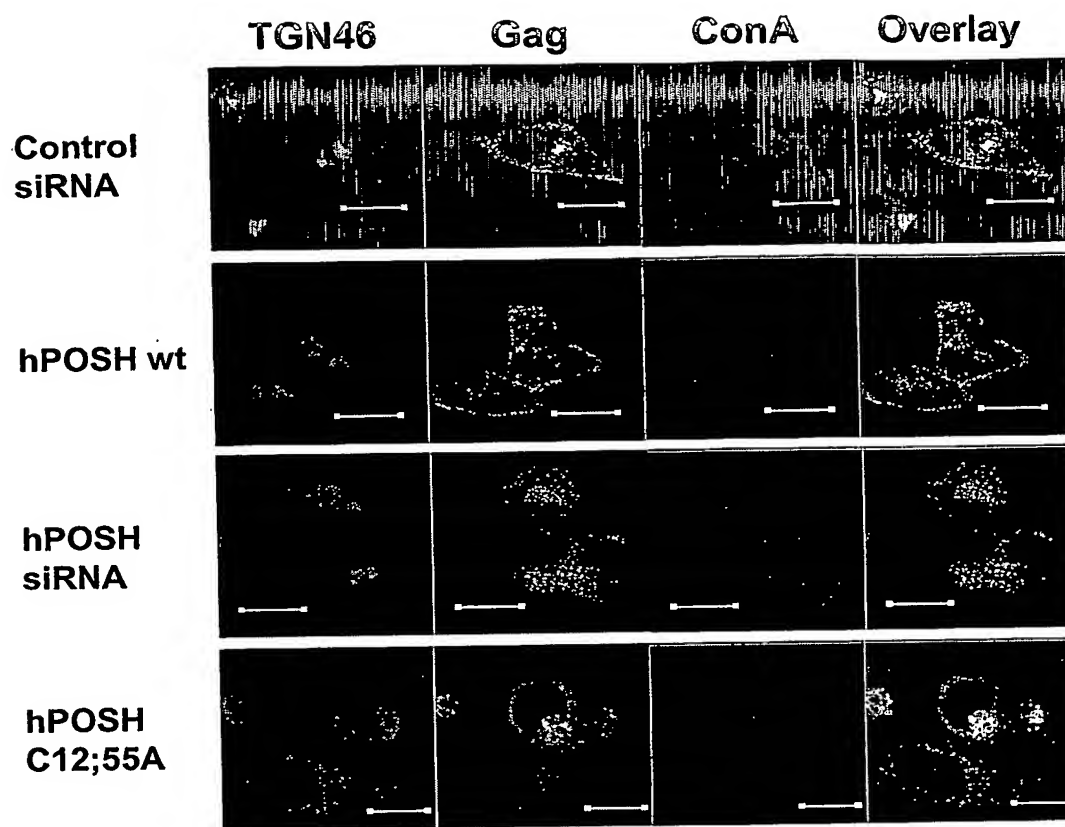




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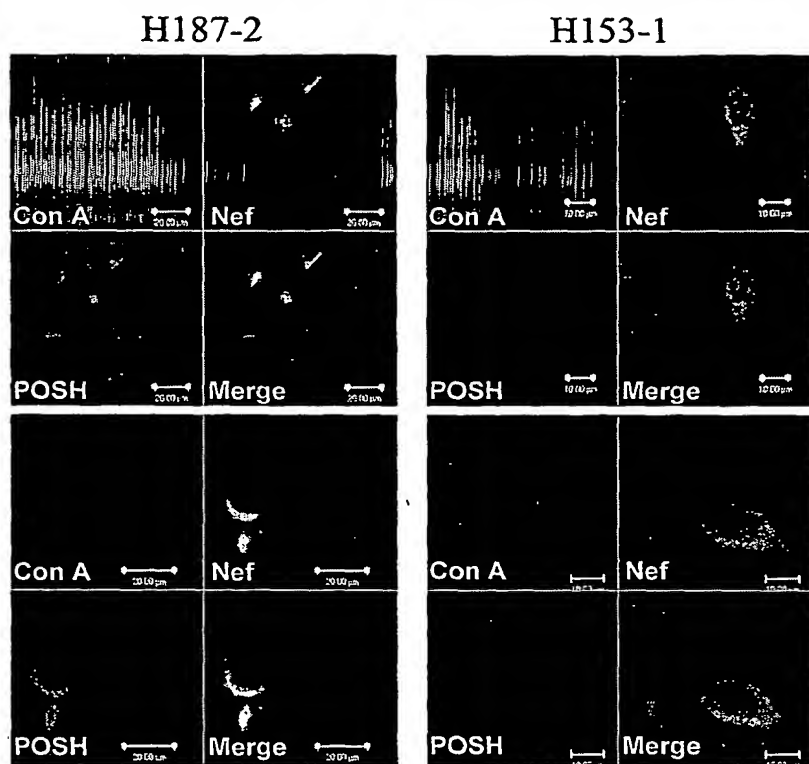


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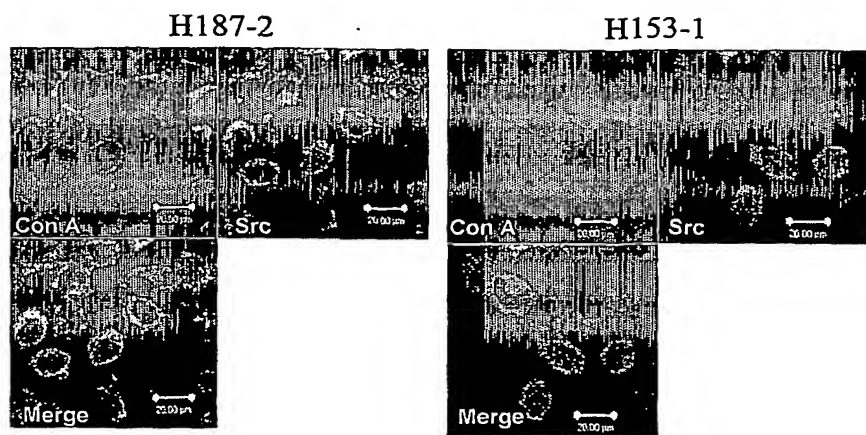
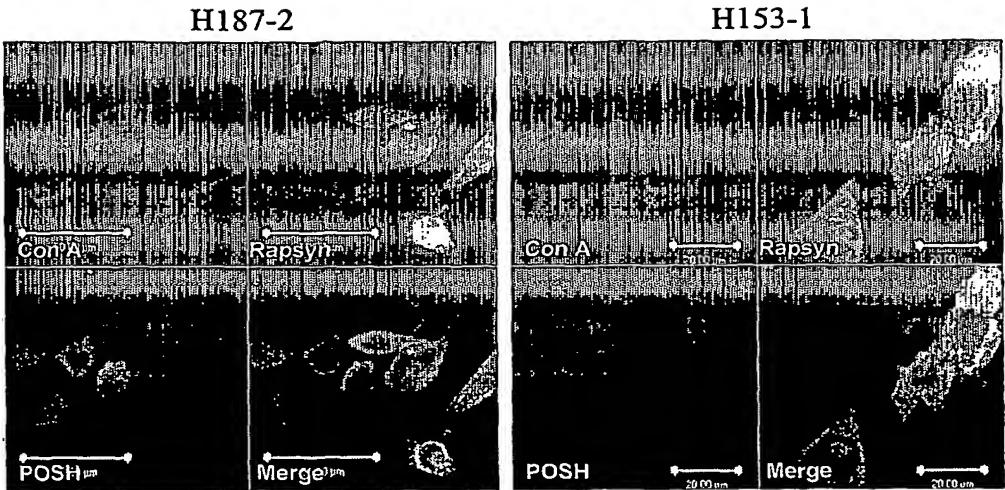


Figure 25.



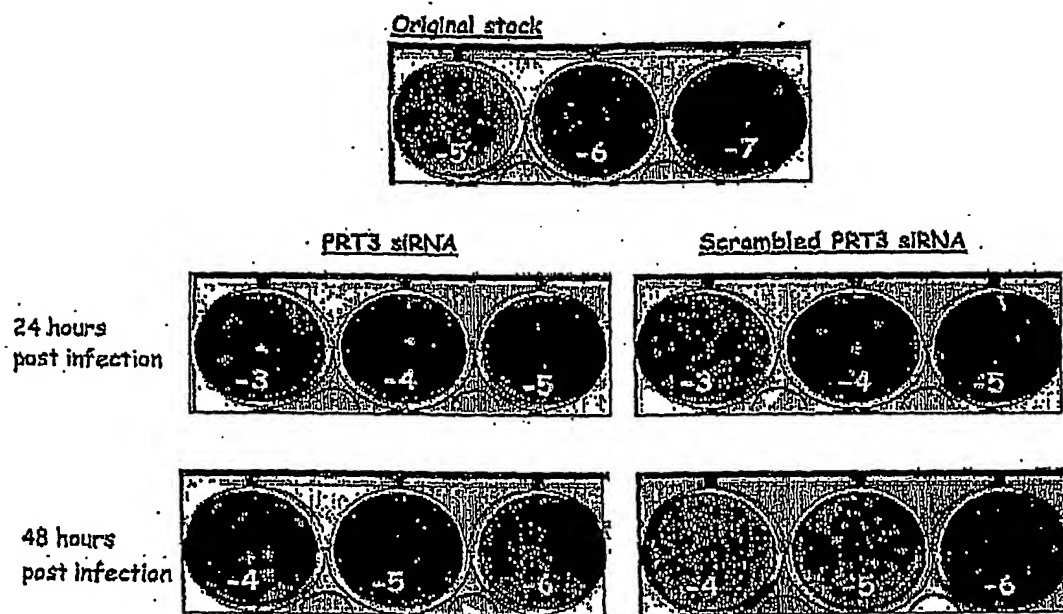


FIGURE 26

Figure 27.

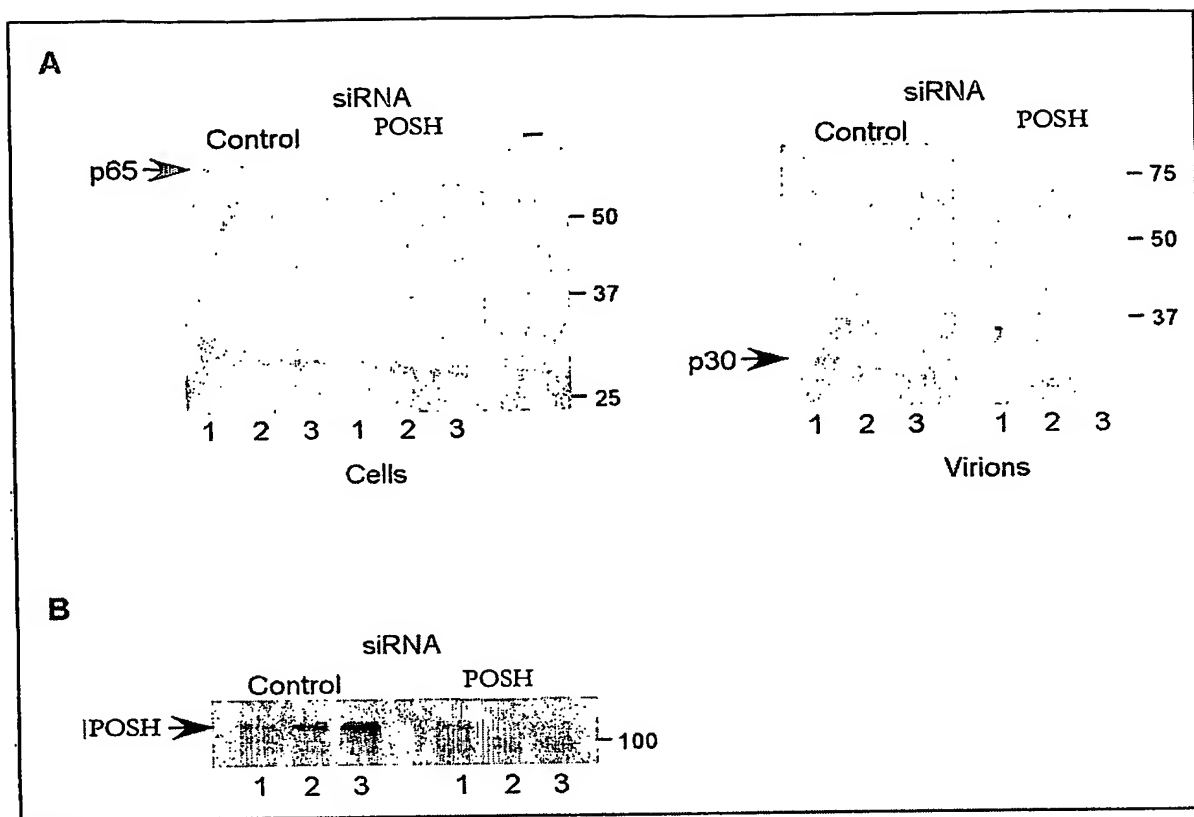


Figure 28.

SiRNA-Tsg101

SiRNA-POSH

Control

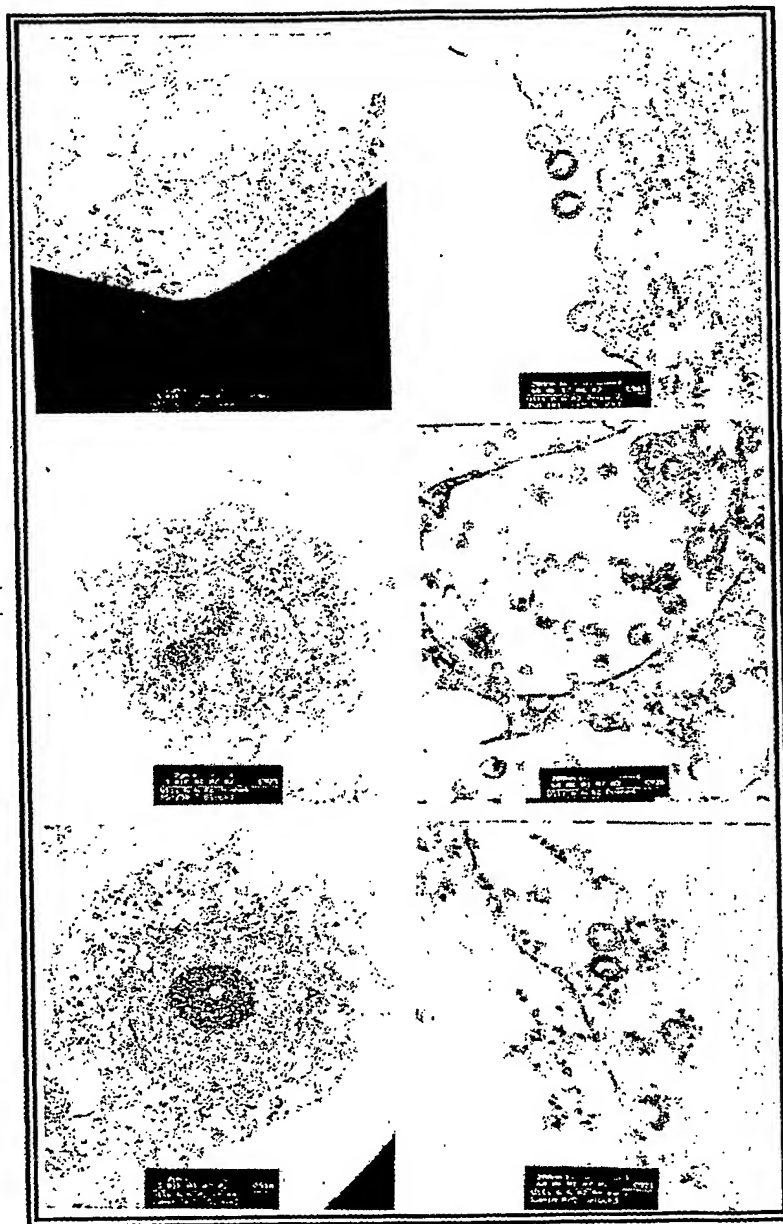


Figure 29.

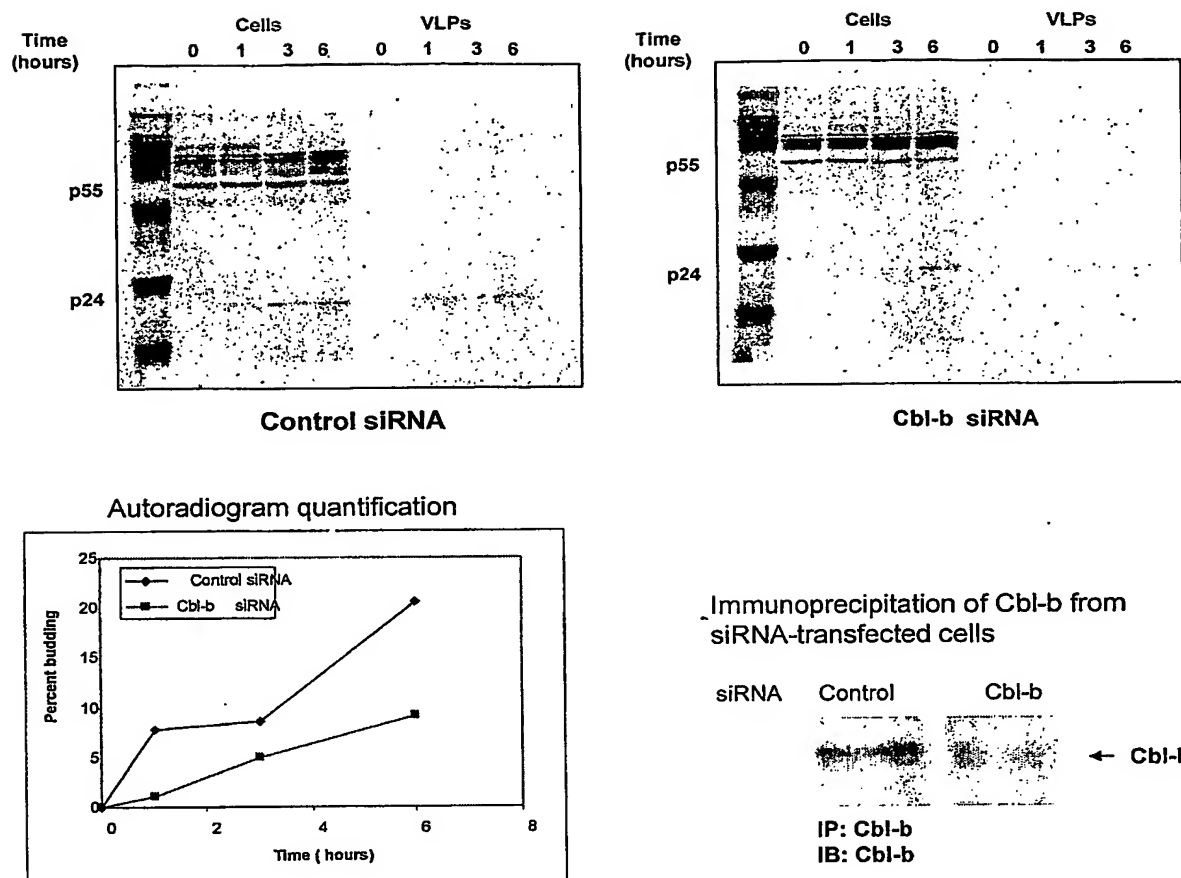


Figure 30.

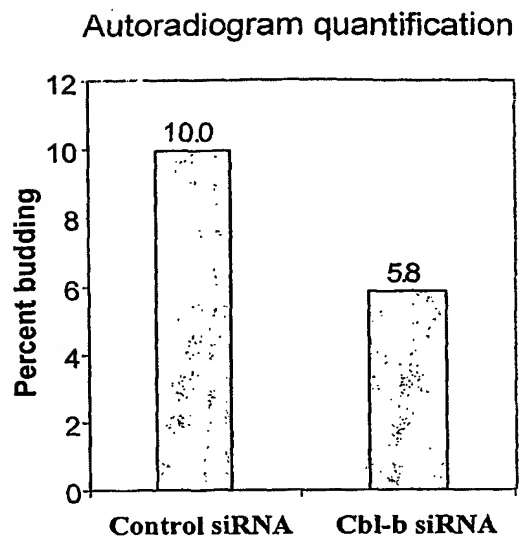
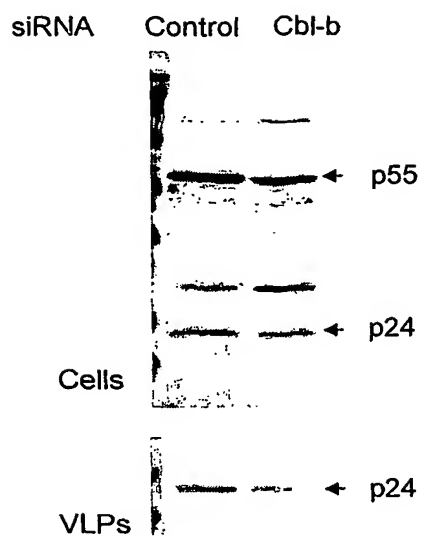




Figure 31. RT activity in VLP secreted from cells treated with indicated siRNAs.

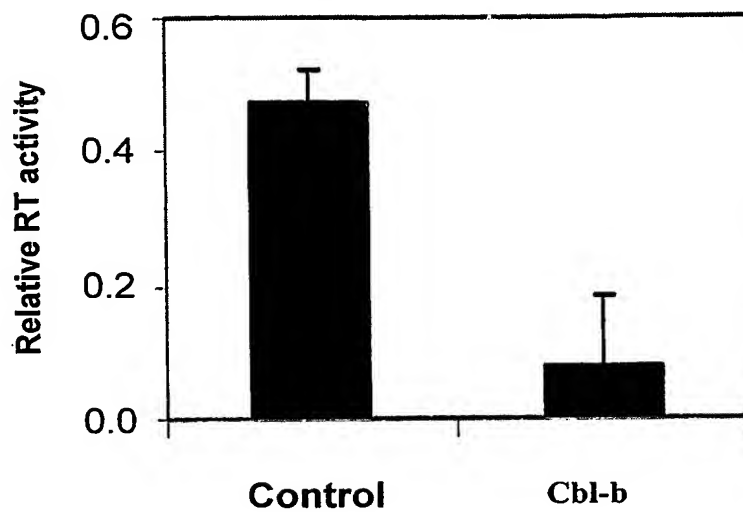
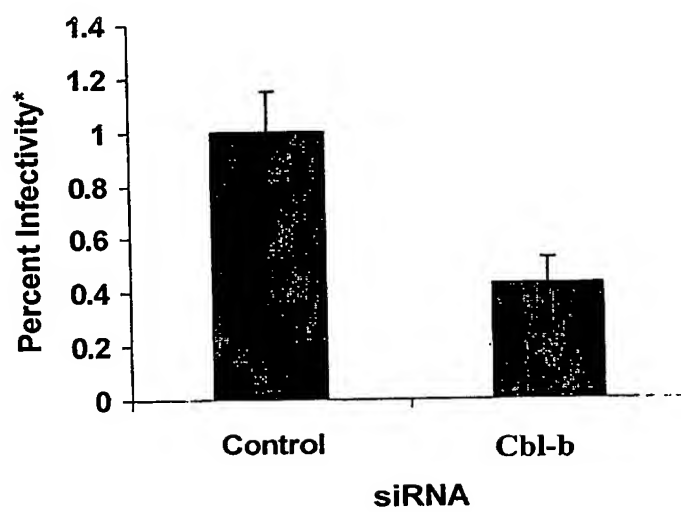


Figure 32. HIV-1 infectivity assay in cells treated with siRNA against Cbl-b.



\* (normalized to control)

Figure 33. RT activity in VLP secreted from cells transfected with indicated plasmids.

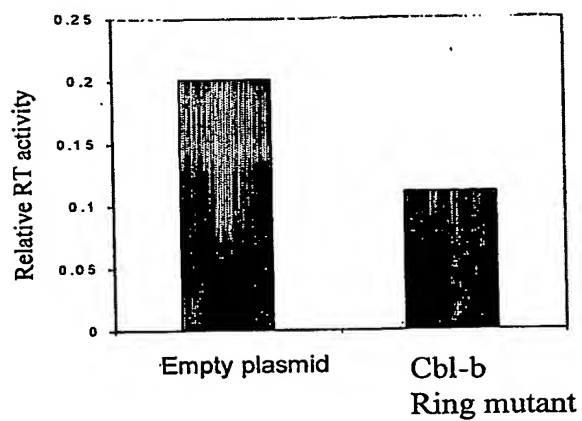
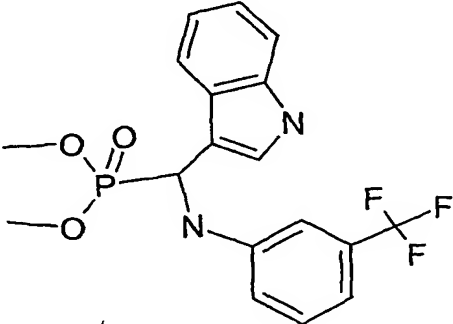
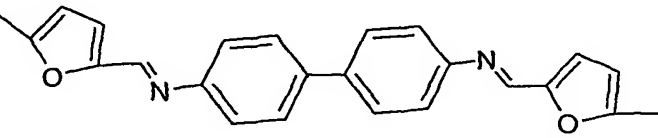
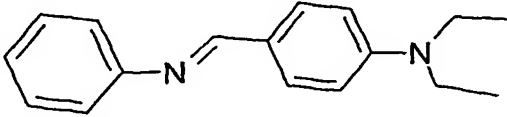
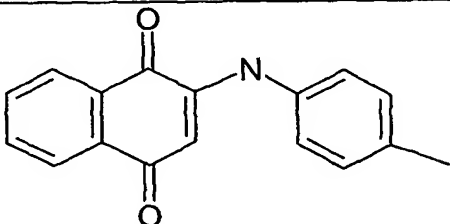
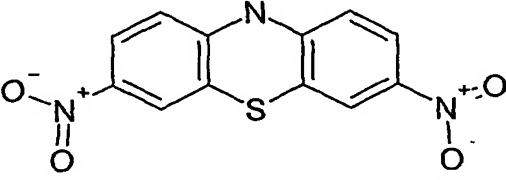
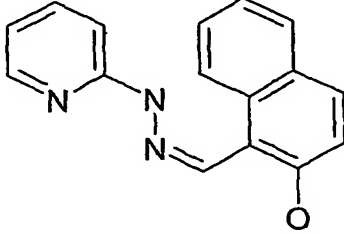
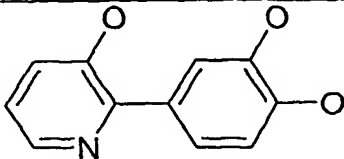
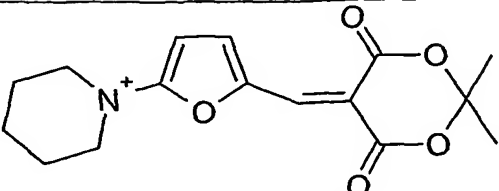
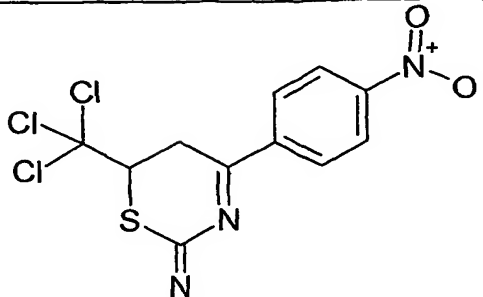
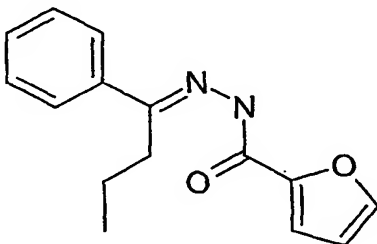
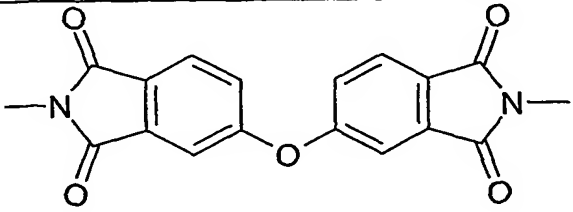
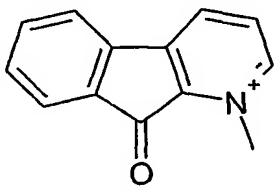
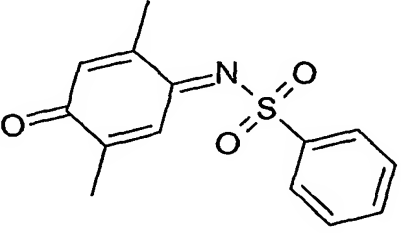


Figure 34.

CAS	MW (gr/mol)	Structure	%A
412945-52-9	398.33		48.7928
52686-41-6	368.44		36.7342
38536-86-6	252.36		25.4204
57182-49-7	263.3		33.8353
63245-76-1	289.27		5.22885
120999-01-1	263.3		42.2514
126324-76-3	203.2		25.1252

164399-38-0	386. 25		35.0247
324526-59-2	352. 63		20.7212
295345-11-8	256. 31		37.633
no cas	336. 31		37.2901
325958-44-9	323. 14		27.7748
88680-99-3	275. 33		23.2871

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